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(54) Title: LONG-ACTING TREATMENT BY SLOW-RELEASE DELIVERY OF ANTISENSE OLIGODEOXYRIBONUCLEOTIDES FROM BIODEGRADABLE MICROPARTICLES

(57) Abstract

Alteration of gene expression in animals by introduction of a biodegradable microparticle composition of an antisense oligodeoxyribonucleotide within a biodegradable polymeric matrix into a target cell is provided. Methods for the controlled delivery of the composition are provided.

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# TITLE

LONG-ACTING TREATMENT BY SLOW-RELEASE DELIVERY OF ANTISENSE OLIGODEOXYRIBONUCLEOTIDES FROM BIODEGRADABLE MICROPARTICLES

## FIELD OF THE INVENTION

This invention relates to a method of controlling gene expression in animals and humans by antisense oligodeoxyribonucleotides, and to slow-release biodegradable polymeric microparticles containing antisense oligodeoxyribonucleotides.

## BACKGROUND OF THE INVENTION

It is known that oligodeoxyribonucleotides (oligos) inhibit the expression of genes through both specific and non-specific mechanisms. For example, treatments with oligos specifically inhibit the growth of cancer cells by blocking oncogene expression. See Watson et al., Cancer Res., 51:3996-4000 (1991). The targeted oligo reacts with DNA or RNA to inhibit expression of a genetic characteristic. The antisense oligo can bind to single-stranded RNA or bind to double-stranded DNA to form triplexes. See U.S. Patent No. 5,190,931. Because anti-sense oligos bind with high specificity to their complement, selectivity can be good and toxic side effects should be minimal. Antisense oligos also are known to inhibit the replication of infectious micro-organisms by blocking the expression of genes critical to their survival. See Han et al., PNAS, 88:4313-4317 (1991).

Currently, most uses of antisense oligos as drugs are via topical administration. No effective delivery system for systemic administration of antisense nucleic acid drugs has been found. Without an efficient and effective delivery system, use of these antisense compounds for most purposes is impractical due to the high concentrations required to achieve the desired

therapeutic effect, coupled with the high cost of these antisense compounds.

The current cost for synthesizing 1 g of antisense phosphorothioate oligo is $18,000 (Synthecell Corp., Rockville, MD). The concentration of oligo required for effective treatment is high. For example, *in vitro* inhibition of tumor cells requires 5-10 mM concentrations. See Watson, et al., Cancer Res., 51:3996-4000 (1991). At this concentration, treatment of a human could require several milligrams of drug. Also, when administered systemically, the half-life of the oligo in circulation is very short. Ninety percent of phosphodiester oligo is removed from the blood stream within one half hour of administration. The effective treatment life of the drug is very short; the treatment is very inefficient. Because of these factors, a one day supply (20 g) of an antisense drug could cost up to $37,500. See Rothenberg et al., JNCI, 81:1539-1544 (1989).

The use of biodegradable microparticles containing drugs as a slow release delivery system is known. U.S. Patents Nos. 4,389,330, 4,919,929 and 4,542,025 disclose a variety of microparticles, or microcapsules, their preparations and their usage. These patents are incorporated herein and made a part hereof. European Patent Application 248,531 discloses RNA and/or DNA or antisense RNA in microcapsules for inhibition of viral replication by the induction of interferon production.

There is a need for a delivery system for antisense oligodeoxyribonucleic acid drugs that extends its useful life in the treated animal. Such a system would prevent the premature degradation of the oligo, and in general gives a long duration, substantially constant dosage treatment that enhances the efficient usage of the drug, putting its cost within a much more reasonable price

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range. Such a system would not rely on interferon induction to inhibit gene expression related to illnesses and control of natural product production in animals.

## SUMMARY OF THE INVENTION

The invention comprises a microparticle composition suitable for the controlled release of an antisense oligodeoxyribonucleotide to a target cell, the microparticle composition comprising: (a) an antisense oligodeoxyribonucleotide present in an amount of about .0001% to 50 wt % of said antisense oligodeoxyribonucleotide based on the weight of an encapsulating matrix of element; (b) a biodegradable polymeric matrix encapsulating the antisense oligodeoxyribonucleotide of elements (a); and (c) the microparticle ranging in diameter from 1 to less than 500 microns. The antisense oligodeoxyribonucleotide is selected from the group consisting of phosphotriesters, methylphosphonate, alkyl phosphotriesters, alpha-anomer, phosphorothioate, diphosphorothioate, methylphosphorothioate, phosphodiester oligodeoxyribonucleotide. The antisense oligodeoxyribonucleotide is conjugated by way of chemical bonds with a material which promotes the uptake or transportation to the nucleus of the target cell of the antisense oligodeoxyribonucleotide, the material selected from the group consisting of proteins, intercalating agents, antibodies, cholesterol, cholesterol derivatives, lipids, nucleic acids, and carbohydrates. The polymeric matrix material of said microparticle is selected from the group consisting of poly-d, l-lactic acid, poly-L-lactic acid, polyglycolic acid, copolymers of mixed d, l-lactic acid and glycolic acid, copolymers of L-lactic acid and glycolic acid, copolyoxalates, polycaprolactone, poly (lactic acid-

caprolactone), poly(glycolic acid-caprolactone, casein, albumin, waxes, polyanhydrides and polyorthoesters.

The invention also comprises a method for the controlled delivery of an antisense oligonucleotide into the target cells of an animal to alter gene expression, the method comprising: (a) coating an antisense oligonucleotide onto a microcarrier of inert, dense material of a size less than the target cell size; (b) encapsulating the coated microcarrier of step (a) within a biodegradable polymer matrix, the encapsulating step forming a microparticle composition; (c) coating the microparticle composition of step (b) onto a planar carrier sheet; and (d) discharging the microparticle composition with a force so that the carrier sheet is restrained from hitting the animal cells, but the microparticle composition is introduced into the target animal cell from which site it can interact with the nucleus of the target cell.

The invention also comprises a method for the controlled delivery of an antisense oligodeoxyribonucleotide into the cells of an animal to alter expression of a target gene, the method comprising:

(a) encapsulating an antisense oligodeoxyribonucleotide within a biodegradable polymer matrix, the encapsulating step forming a microparticle composition;

(b) containing the microparticle composition of step (a) in a dosing device suitable for delivery of the microparticle to a target cell; and

(c) discharging the microparticle composition from the dosing device of step (a) into an animal from which site the antisense oligodeoxyribonucleotide contained within the biodegradable polymer matrix can interact

with the target cell upon degradation of the polymer matrix.

The invention also comprises a method for inhibiting the expression of genes comprising:

introducing into a target animal tissue a controlled-release, biodegradable microparticle composition comprising:

(a) an antisense oligodeoxyribonucleotide specific to the gene expression to be inhibited and present in an amount of about 0.0001 wt. % to 50 wt. % based on the weight of an encapsulating polymeric matrix of element (b);

(b) a biodegradable polymeric matrix encapsulating the antisense oligodeoxyribonucleotide of element (a); and

(c) the microparticle ranging in diameter from 1 to 500 microns.

The antisense oligodeoxyribonucleotide is selected from the group consisting of phosphotriesters, methylphosphonate, alkyl phosphotriesters, alpha-anomer, phosphorothioate, diphosphorothioate, methylphosphorothioate, phosphodiester oligodeoxyribonucleotide.

The nucleic acids are conjugated by way of chemical bonds with molecules which promote the uptake or transportation to the nucleus, the molecules selected from the group consisting of fatty acids, phospholipids, sphingolipids, glycolipids, triglycerides, gangliosides, steroid hormones, cholesterol, peptidoglycans, lectins, single stranded or double stranded RNA, DNA, and intercalating agents.

The polymeric matrix containing the oligodeoxyribonucleotide is coated with molecules which promote the uptake of the nucleic acid into the cell

and/or transportation to the nucleus, the molecule selected from a group consisting of proteins, intercalating agents, nucleic acids, lipids, or carbohydrates.

The polymeric matrix material of the microparticle is selected from the group consisting of poly-d, l-lactic acid, poly-l-lactic acid, polyglycolic acid, copolymers of mixed d,l-lactic acid and glycolic acid, copolymers l-lactic acid and glycolic acid, copolyoxalates, polycaprolactone, poly (lactic acid-caprolactone), poly (glycolic acid-caprolactone), casein, albumin, and waxes.

## DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a low cost, efficient and therapeutically effective delivery system for antisense oligodeoxyribonucleic acid drugs (oligos) and modified forms thereof. The oligos are delivered into animals for the inhibition of gene expression as it relates to illness and control of natural product production in animals. The oligos are in compositions comprising microparticles containing the drug in a matrix of non-toxic biodegradable polymer.

In the method of the present invention, antisense oligodeoxyribonucleic acid, encapsulated in a microparticle of a non-toxic biodegradable polymer matrix, is delivered into the cells of an animal to be treated systemically by injecting, Biolistic® particle delivery, implants and other known means. This method is termed Controlled-release Antisense Technology with Oligodeoxyribonucleotides (CATO). The CATO delivery system enables treatment of humans or other animals, in which certain natural products can be produced by altering the expression of one or more genes. Illness involving over-expression or inappropriate expression of certain genes critical to pathogenesis, can be treated

by inhibition or enhancement of gene expression with a lower total dose of oligo than if treated systemically without controlled release. The microparticle drug can be injected, bombarded or implanted directly into a tumor. Alternatively, the microparticle drug can be released in one site (such as the peritoneal cavity) and be taken up by cells or tissue at a distant site. Additionally, the CATO technology can be applied to extra cellular body materials such as blood, interstitial fluids and brain ventricles. The invention also relates to the composition of the biodegradable microparticles containing the oligo. By adjustment of the composition of the polymer matrix, the rate of release of the drug can be predetermined and so optimized. The drug will be available and released at the site needed for biological activity for an extended length of time as the matrix degrades, and the matrix will protect the unexposed drug from degrading before it has effected treatment.

The present invention offers the advantage of durations of action ranging from only 30-60 days to more than 200 days depending upon the type of microsphere selected. In the preferred embodiment the microparticles or microspheres are designed to provide treatment in animals over a period of 30 days. The duration of action can be easily controlled by manipulation of the polymer composition, polymer:drug ratio, and microsphere size. Surface coating of the microparticle can also be used to promote controlled release. The compositions of the present invention are microparticles (microcapsules), prepared by conventional techniques, having a biocompatible, biodegradable polymeric matrix with oligo distributed within the matrix. The microparticles can be of any conventional type and may include other pharmaceutically effective

ingredients, such as an antibiotic, vaccines, or peptide, and other conventional additives.

As part of the description of the invention, the following words and terms are used. The term "antisense" as applied to compounds herein refers to nucleic acid binding drugs that control gene expression and which happen to be DNA molecules. "Antisense" also refers to the method of using an oligonucleotide having a base sequence substantially complementary to a portion of messenger ribonucleic acid to control or inhibit synthesis of an organism's biological component coded for by the ribonucleic acid. The term "biocompatible" as used herein means non-toxic to the human body, non-carcinogenic and non-inflammatory in body tissues. The matrix material is biodegradable in the sense that the polymeric material degrades by bodily processes to products readily disposable by the body that do not accumulate excessively in the body. The biodegraded products also are compatible with the body.

The term "controlled release" as used herein means the slow and consistent systemic release of an active compound (oligo) over a period of time.

The term "oligo" as used herein means antisense oligodeoxyribonucleotides.

The term "microparticles" as used herein means particles either solid or of the reservoir type which contain an active agent, herein an oligo, either in solution or in crystalline form. The active agent is either dispersed or dissolved within the polymer which serves as the matrix of the particle, or is contained within the polymer in reservoir fashion with the polymer serving as the outer wall of the reservoir.

The term "animal" is used herein is its usual biological connotation, and encompasses all species of animals large enough to be treated, particularly humans,

food animals, and mammals in general; birds, fish and the like are also included.

The term "food animal" means any animal that is consumed as a source of protein in the diet of humans or other animals. Typical food animals include bovine animals, for example cattle; ovine animals, for example sheep; porcine, for example pigs; fowl, for example chickens and turkeys; rabbit and the like. The terms "control" and "inhibit" as used herein as applied to an illness mean the prevention, curing, arrest, or other beneficial pharmacological effect on the illness.

The terms "administered" or "delivered" mean any method of delivering the CATO microparticles to an animal, such as, for example, parenteral (intravenous, intramuscular, or subcutaneous), nasal, vacular, tracheal or oral administration and also encompasses particle delivery means.

The term "particle delivery" means any method of bombardment of microparticles to any animal tissue, but particularly the method disclosed in U.S. Patent No. 4,945,050.

The term "illness" as used herein means a malady to the species caused by internally or externally originating entities. An illness may be localized, such as some tumors, or it may be wide-spread throughout the animal body as in many viral diseases and metatastic cancers. Illnesses of particular significance to the present invention are cancer and infectious, genetic and auto-immune diseases.

The term "locale of the illness" refers to a localized illness and means the zone location of the illness.

The oligos useful in the process and compositions of the present invention are known in the art and in general are synthetic, although some could possibly be

obtained without synthetics. The particular choice of oligo is, of course, dictated by the effect desired.

The preferred oligos of the process and composition of the present invention are synthetic phosphorothioate, methylphosphorothioate, alkyl phosphotriesters, and alpha-anomer oligos, and derivatives thereof. Oligos can be conjugated with proteins, intercalating derivates, lipids, carbohydrates and other nucleic acids that promote the uptake of the oligo by the cell or otherwise increase the oligo's activity.

Synthetic oligos have been used successfully to inhibit DNA replication, retroviral replication, pre-mRNA processing and protein synthesis with high specificity. These oligo-mediated inhibitory effects have been observed by analysis of specific RNA and protein synthesized by cells, and by monitoring the enhancement or suppression of cellular growth inhibition, morphological changes or reduction in viral infectivity. The mechanisms by which oligos inhibit these activities include: 1) hybridization of the antisense oligos to the target RNA molecule which prevents ribosomal complex assembly or mRNA translation, 2) RNase H-mediated degradation of the resultant DNA/target RNA duplex, 3) inhibition of pre-mRNA splicing, 4) formation of covalent bonds with a target RNA by using chemically reactive oligos; or 5) triple helix DNA formation.

The benefits of using synthetic DNA oligos in antisense studies are: 1) Retroviral transfection is not required for cellular uptake to occur. Oligos are actively transported into some cells when directly included in the cell culture media. Oligos can also be microinjected or bombarded directly into cells; 2) RNase H-mediated degradation of the target RNA occurs in the stable oligo/target RNA duplex, unlike RNA-RNA duplexes

as occurs in antisense RNA treatment; 3) Antisense oligos bind selectively to their target sequence; 4) Use of oligos offers control of the copy number of antisense molecules in the cell, an important factor in suppression of gene expression; 5) Oligos can be modified during the synthesis process. Modified oligos have been shown to be more effective at inhibition of gene expression in some systems; 6) Oligos can be specifically synthesized to bind to selective targets reducing possible side effects; 7) Specific ligands can be attached to the oligo increasing cellular uptake or causing uptake by specific cells.

Early antisense studies utilized unmodified, synthetic phosphodiester oligodeoxynucleic acids. However, because of their charged phosphate backbone and increased lipophobicity, phosphodiester oligos could not be easily transported into the target cells. Modified phosphodiester oligos have been synthesized and tested to inhibit gene expression. Oligos have been modified at either their phosphate backbone, deoxyribose, or nitrogenous bases.

Antisense oligos with modified backbones are:

1) Oligodeoxyribonucleotide Methylphosphonates. Methylphosphonates are uncharged oligonucleotides in which the phosphate oxygen is replaced by a methyl group. Methylphosphonates are more stable than phosphodiester oligos because they have increased resistance to 3' and 5' exonuclease, a naturally occurring nuclease which breaks down DNA in the body. Methylphosphonates may be able to enter cell membranes more easily than phosphodiester oligos because of increased lipophilicity.

2) Oligodeoxyribonucleotide O-alkylphospho-triesters. O-alkylphosphotriesters are a neutral DNA analog, in which the charged oxygen moiety is alkylated,

and are therefore more efficient at being transported into the target cell. The alkyl group can be any organic molecule.

3) Oligodeoxyribonucleotide Phosphorothioates. Phosphorothioate DNA oligo form stable hybrids with both DNA and RNA, are substrates for RNase H, are resistant to many nucleases and can inhibit nucleic acid synthesis catalyzed by DNA polymerases and certain reverse transcriptases. Because of their increased hydrophobicity, they enter cells more easily than phosphodiester oligos.

4) Oligodeoxyribonucleotide phosphoramidates - contain internucleotide nitrogen-phosphorous bonds. Homo-oligomer phosphoramidates also belong to this class of oligo. The oligo/RNA complex formed with this class of oligo is not as stable as that formed with phosphodiester oligos.

5) Oligodeoxynucleotide methylphosphonothioate - contain internucleotide sulfur-phosphate and methyl-phosphate bonds. An example of a sugar-modified oligo is the alpha-anomer. Alpha-anomeric DNA is an analog of phosphodiester oligo in which the position of the hydrogen at the 1' position of the deoxyribose ring is opposite to that found in normal or Beta-DNA. Alpha-DNA oligomers are degraded less rapidly than phosphodiester oligos.

Various chemical modifications at either the 3'- and/or 5'-termini or within individual nucleic acid bases have been shown to improve the antisense potential of synthetic oligomers described above. Some of the chemical moieties are: the acridine derivatives, bathophenanthroline-Ru(II) complexes, DANSYL, MANSYL, AEDANS-dUTP, and poly-L-lysine.

"Reactive oligos" stabilize the interaction of the complementary oligonucleotides with their specific RNA

or DNA targets, and may also induce damage to the target messenger RNA. Molecules attached to oligos which result in "reactivity" include intercalators (e.g. acridine, phenazium), photochemically activated cross-linking (proflavin, porphyrin derivates, azido derivatives, furocoumarin) or cleaving (e.g., methyl-porphyrin XXI) agents, alkylating agents (e.g. chloroalkylaminoaryl), or redox active nucleic acid cleaving groups (e.g., 10-Cu(II)-phenanthroline, EDTA-Fe, porphyrin-Fe).

Attachment of lipophilic moieties such as cholesterol or long-chain alkyl groups at the 3' or 5' termini of oligos increases the hydrophobicity of the oligomer, promoting enhanced uptake through the cell membrane. These modifications generally potentiate the antisense effect mediated by natural or modified oligomers.

Circular synthetic oligo sequences can bind more strongly than analogous linear sequences. These circular sequences may be more resistant to exonuclease digestion. Also, they may selectively target viral and messenger RNA.

Conjugation of DNA to proteins and peptides has been an effective method for targeting the delivery of genes to certain cell-types. For example, Asialo-glycoprotein conjugated to a plasmid via poly-l-lysine is taken up in liver cells and expressed. Likewise, antibodies specific for certain cellular ligands can be coupled to oligos to promote their targeted uptake into specific cells. Receptor mediated endocytosis could be accessed by coupling ligands, such as transferrin to the oligos or polymers. These combinations will increase specific uptake.

The illnesses which the present invention is most useful in controlling are those which involve the

overexpression or inappropriate expression of certain genes. Examples of these illnesses are listed below.

1. Cancers. Proto-oncogenes are genes which are normally expressed in developing cells but are also inappropriately expressed in neoplastic cells. Oncogenes have been shown to play a pivotal role in the pathogenesis and propagation of some cancers such as neuroblastoma, lymphomas of T-cell and B-cell origin.

2. Infections. Micro-organisms colonizing a host contain genes specific to their species, the expression of which are important for their survival. When animals have been infected with micro-organisms which display a defined pathologic lesion (such as herpes simplex virus-associated stromal keratitis) and treated with antisense oligos specific for those critical genes, they inhibit the pathology associated with this infection. It is believed that diseases associated with infection by any virus, bacteria, protist, parasite, or fungus can be inhibited by the CATO antisense therapies.

3. Autoimmune disease. Certain genes have been shown to be overexpressed in diseases such as rheumatoid arthritis, systemic lupus erythematosus, psoriasis, and multiple sclerosis, among others. These genes are targets for inhibition by CATO antisense oligos treatment.

Treatment of Human, Drug-Resistant Tumors with CATO

An antisense oligonucleotide specific for sequences in the multidrug resistance (MDR) gene (which is amplified and overexpressed in many tumors) (Pastan et al. (1988) PNAS, 85:4486) is covalently conjugated to a ligand which binds to a cell-surface receptor found on a cancer cell, for instance, the estrogen or progesterone receptor on breast adencarcinoma cells. This conjugate is microencapsulated and administered to the patient as an adjunct to chemotherapy and provides continuous

release of the oligo for homing and uptake by the tumor cells. Applicants predict that by so treating the tumor cells, the MDR gene would be shut down, which would allow for more effective treatment with antitumor agents. Overexpression of the MDR gene in tumor cells dramatically decreases the efficacy of tumoricidal agents, such as methotrexate, by transporting the anticancer compound out of the tumor cell once it has entered.

In a preferred embodiment, the oligos are administered to humans or other animals, particularly food animals, by a single administration of the oligo-loaded microparticles. The microparticles release the active oligo in a constant or pulsed manner into the animal and eliminate the need for repetitive injections.

In addition, the preferred embodiment allows for the localized treatment of certain diseases. Treatment of tumors by injection of controlled-release oligos specific for the malignancy of the tumor cell will inhibit the growth and metastasis of the tumor. In the case of rheumatoid arthritis, an autoimmune disease which is localized to the joints, localized treatment with controlled-release antisense therapeutic oligos specific for a cytokine gene important for maintaining the chronic state of the disease may be beneficial.

The preferred embodiment also enables treatment of animals with oligos to inhibit the expression of genes causing the animal to synthesize products altered in composition from those naturally produced. The inhibition of the gene encoding a cholesterol synthesis enzyme might cause chicken egg to be produced containing a lower concentration of cholesterol.

The formulations of the present invention contains oligo dispersed in a microparticle matrix material. The preferred construction of microparticles of the

invention are described in detail in U.S. 4,389,330, U.S. 4,919,929, and U.S. 4,530,840. The microparticles of the invention are composed of a polymer which is, preferably an aliphatic polyester such as either a homopolymer or copolymer of lactic or glycolic acids. Other degradable polymers may be used, such as, for example, polycaprolactone, polydioxonose, polyorthoesters, polyanhydrides, and natural polymers including albumin, casein, and waxes. The amount of oligo incorporated in the microparticles usually ranges from less than 0.00005 wt% to as high as 75 wt%, preferably 0.0001 to 50 wt%. By "weight %" is meant parts of oligo per parts of polymer by weight. For example, 10 wt% would mean 10 parts oligo per 90 parts polymer by weight.

The polymeric matrix material of the microparticles in the present invention must be biocompatible and biodegradable polymeric material. Suitable examples of polymeric matrix materials include poly (glycolic acid), poly (d,l-lactic acid, poly (l-lactic acid) and copolymers thereof, copolyoxalates, polycaprolactone, poly (lactic acid-caprolactone), and the like. Suitable polymeric materials also include waxes such as glycerol mono-and distearate and casein.

The molecular weight of the polymeric matrix material is of some importance. The molecular weight (mw) should be high enough so that it forms satisfactory polymer coatings, i.e., the polymer MW is proper for the polymer to be a good film former. Usually, a satisfactory molecular weight is greater than 5,000 daltons. The various film-forming polymer compositions have molecular weights readily determined by known techniques. Also, the polymer molecular weight also plays a significant role (along with composition, purity, optical form, etc.) in rate of degradation. In

general, the higher the MW the slower the degradation. Average molecular weights of about 500,000 are preferred.

Oligo release from the particle can also be by leaching through the polymer matrix. In this method the oligo is released before the polymer is significantly degraded or simultaneously with polymer degradation. By an appropriate selection of polymeric material, a microparticle formulation can be made such that the resulting microparticles exhibit two phases of release properties. This and manipulation of oligo/polymer ratio are useful in affording multiphasic release patterns.

The microparticle products of the present invention can be prepared by any method capable of producing microparticles in a size range acceptable for use in an injectable composition. Generally, microencapsulation processes are classified according to the principal types: (1) phase-separation methods including aqueous and organic phase separation processes, melt dispersion and spray drying; (2) interfacial reactions including interfacial polymerization, *in situ* polymerization and chemical vapor deposition; (3) solvent extraction method; and physical methods, including fluidized-bed spray coating, multi- and single-orifice centrifugal coating, electrostatic coating and physical vapor deposition.

A preferred method of preparation is the method described in U.S. 4,919,929. Phase separation methods, as the term implies, rely on differential solubility characteristics that cause a wall- or shell-forming matrix material to separate from solution or suspension and deposit around particles or droplets of the substance to be encapsulated. The separation, itself, may be brought about physically, as by the addition of a

non-solvent or by a change in temperature, or chemically, as by a change in pH.

Organic phase-separation processes usually employ a dispersion or an emulsion of oligo in a solution or a high-molecular-weight polymer in an organic solvent. To this mixture is added a non-solvent or liquid polymer that causes the high-molecular-weight polymer to separate from solution and collect as a shell around the suspended therapeutic agent(s). The shell, still swollen with solvent, is then hardened by a further addition of non-solvent or by some other process that strengthens the shell and improves the barrier properties, controlling release by its oligo permeability and/or degradation rate.

Typically, an aqueous solution or suspension of a lipophobic antisense oligonucleotide is added to a non-aqueous solution of a suitable matrix polymer, and the mixture is agitated to cause the formation of a water-in-oil emulsion. Depending upon its solubility in water, the agent may be present at a concentration of 0.1 to 50% in the aqueous phase, which preferably is 0.1 to 20% by weight of the total mixture. The external organic phase may contain 5 to 10% of the matrix polymer. Usually, however, the ratio of agent in the internal phase (aqueous solution or suspension) to polymer is to 1:4.

An aqueous phase separation process employs a dispersion or an emulsion of a water-insoluble therapeutic substance in an aqueous solution or dispersion of a polymer. The polymer is caused to separate as gel particles; these collect around the therapeutic agent to form a shell; the shell is hardened; and the microparticles are isolated. In the coacervation process, which is the most common of the aqueous phase-separation processes the water-soluble

therapeutic agent, which may be in the form of particles or droplets, is dispersed in an aqueous sol of a hydrophilic colloid which becomes ionized in water; a second sol of opposite charge is added; and the mixture is caused to gel by a dilution with water, an addition of salt, an adjustment of pH, or a change in temperature, or any combination of these procedures. Appropriate conditions of coacervation are determined readily by routine trial because the various usable polymers differ significantly in physical and chemical properties according to source and method of isolation or preparation. A region of coacervation is determined by combining solutions or sols of two polymers at various concentration, temperatures, and levels of pH, and observing the conditions required for gelation. From these determinations can be drawn a ternary phase diagram, showing the area of compatibility and the region of coacervation, at a given temperature and pH. The changes in concentration, temperature and pH to effect gelation are then apparent.

Each preparation of microparticles requires careful control of conditions, and somewhat different conditions are required for various material being encapsulated. The degree of agitation, for example, affects the size of emulsion droplets. The surface properties of the droplets may require alterations in the procedures to insure substantially complete deposition of matrix material about the droplets and to minimize formation of particles not participating in microencapsulation. The volume of water added in the dilution step is not critical, but generally larger volumes are required to maintain a stable emulsion when larger droplets are encapsulated.

The above phase separation can be adapted to an alternate technique in which the first step of forming a

stable emulsion or suspension of an oligo is accomplished by dispersing the oligo in a solution of the matrix material. Thereafter, the emulsion is added drop-wise to a non-solvent with stirring to precipitate the polymer coating material to form microparticles containing the oligo.

Another type of phase separation technique is the melt-dispersion microencapsulation technique. A heat-liquifiable, waxy coating material, preferably of a low-melting wax such as glycerol distearate, is suspended in an inert liquid such as a silicone oil or a fluorocarbon in which neither the wax nor the oligo is appreciably soluble. The mixture is heated and stirred vigorously to melt and emulsify the wax. The oligo is powdered and screened to the desired size range and the waxy coating material is dispersed with high shear agitation. The liquefied wax coats the oligo to form the waxy liquid-coated microparticles. Thereafter, the formed microparticles are solidified by continued agitation which cools the particles. The microparticles are then isolated by filtration and dried as described earlier.

Another method of forming the microparticles is by interfacial microencapsulation. This involves bringing two reactants together at a reaction interface where polycondensation of the reactants, usually monomers, occurs to form a thin, insoluble polymeric film. One technique of establishing the interface for the encapsulation process is the dispersion or emulsification of the oligo with one of the reactants. The reactant forms the condensation polymer in a continuous phase containing the second reactants.

Still another method of microencapsulation is by solvent extraction. In this method, the desired oligo compound is added to the polymer matrix material which has been dissolved in a suitable solvent. The oligo

compound may be soluble or insoluble in the solvent for the polymeric material. Optionally, the oligo may be dissolved or dispersed in a second media fluid by adding it to the polymeric matrix solvent.

The mixture of ingredients in the solvent is emulsified in a continuous-phase processing medium; the continuous-phase medium being such that a dispersion of microdroplets containing the indicated ingredients is formed in the continuous-phase medium. The continuous-phase processing medium, commonly water, and the organic solvent must be immiscible.

Nonaqueous media, such as xylene and toluene and synthetic oils and natural oils can be used as the continuous phase processing medium. Usually, a surfactant is added to the continuous-phase processing medium to prevent the microparticles from agglomerating and to control the size of the solvent microdroplets in the emulsion. A preferred surfactant-dispersing medium combination is a 1 to 10 wt % poly (vinyl alcohol) in water mixture. The dispersion is formed by mechanical agitation of the mixed materials. An emulsion can also be formed by adding small drops of the active agent-wall forming material solution to the continuous phase processing medium. The temperature during the formation of the emulsion is not especially critical but can influence the size and quality of the microparticles and the solubility of the oligonucleotide in the continuous phase. Of course, it is desirable to have as little of the oligo in the continuous phase as possible. During processing, the temperature must not be so low as to make too viscous or solidify the solvent or processing medium. Nor should the temperature be so high as to evaporate too much medium or degrade the oligo. Accordingly, the dispersion process can be conducted at any temperature which maintains stable operating

conditions, which preferred temperature being about 30°C to 60°C, depending upon the oligonucleotide and excipient selected.

The dispersion which is formed is a stable emulsion. From this dispersion the organic solvent immiscible fluid is partially removed in the first step of the solvent removal process. The solvent can easily be removed by common techniques such as heating, the application of a reduced pressure or a combination of both. The temperature employed to evaporate solvent from the microdroplets is not critical, but should not be so high that it degrades the oligo nor should it be so high as to evaporate solvent at such a rapid rate as to cause defects in the wall-forming material. Generally, from 5 to 75%, preferably 1 to 25% of the solvent is removed in the first solvent removal step.

After the first stage, the dispersed microparticles in the solvent immiscible fluid medium are isolated from the fluid medium by any convenient means of separation. For example, the fluid can be decanted from the microparticle or the microparticle suspension can be filtered. Conventional combinations of separation techniques can be used if desired.

Following the isolation of the microparticles from the continuous-phase processing medium the remainder of the solvent in the microparticles is removed by extraction. In this step, the microparticles can be suspended in the same continuous-phase processing medium used in step one, with or without surfactant, or in another liquid. The extraction medium removes the solvent from the microparticles and yet does not dissolve the microparticles. During the extraction, the extraction medium with dissolved solvent must be removed and replaced with fresh extraction medium. This is best done on a continuous basis, where the rate of extraction

medium replenishment is critical. The appropriate rate of extraction medium replenishment of a given process is easily determined. After the majority of the solvent has been removed from the microparticles, the microparticles are dried by exposure to air or by other conventional drying techniques, such as vacuum drying, drying over a desiccant, or the like.

Another method of encapsulation is physical microencapsulation. Physical microencapsulation techniques are characterized by the continuous envelopment of particles or droplets of a substance in a fluid film, as a melt or solution of the coating material, in an apparatus containing coaxially - or sequentially-spaced orifices. Thereafter, the fluid coating is hardened by a standard cooling technique or by solvent evaporation.

Among the physical methods of microencapsulation are those that involve the passage of liquid or solid core material through a liquid matrix material. The stream is disrupted by some means to cause the formation of liquid-coated droplets or particles, and the resulting particles are cooled or otherwise treated to solidify the shell material. For example, an aqueous solution of a substance to be encapsulated is aspirated into rapidly flowing stream of molten glycerol distearate, and the mixture is ejected through a fine nozzle. On emergence from the nozzle, the liquid stream disintegrates into droplets, each consisting of an aqueous core surrounded by liquid wax. As these fall through air, the shells cool and solidify, and microparticles result. In another version of this process, the impelling force is supplied by a rotating member, which ejects the core material centrifugally through the shell-forming liquid.

The variations of these and other processes of microencapsulation are many. As is readily apparent to those skilled in the art, no one process nor any single set of conditions is applicable to all substances, but instead a useful process is chosen and the conditions optimized to achieve the desired results with a specific oligo.

The microparticle products of the present invention are usually made up of particles of a generally spherical shape, although sometimes the microparticles may be irregularly shaped. The microparticles can vary in size, ranging from sub-micron to millimeter diameters. Preferably, diameters of less than 1 to 500 μm are desirable for oligo formulations, which allows administration of the microparticles with a standard gauge needle or other conventional methods.

Prior to administration to an animal or group of animals, the microparticles are suspended in an acceptable pharmaceutical liquid vehicle, and then the suspension is injected into the desired portion of the body of the animal.

The amount of oligonucleotide administered to the animal depends on the particular animal species, target gene sequence, illness, length of time of treatment, age of the animal, and amount of treatment desired as is understood by one skilled in the art.

The microparticles can be mixed by size or by type so as to provide for a delivery of oligos to animals in a multiphasic manner and/or in a manner which provides different oligos to the animal at different times, or a mixture of oligos to the animal at the same time. Other biologically active agents commonly administered to animals may be blended with the oligo formulation. For example, antibiotics, antihelmintics, vaccines, or any desired active agent, either in microparticle form or in

conventional, unencapsulated form may be blended with the oligonucleotide and provided to an animal by the method of the invention.

In a preferred method of preparing microparticles containing an antisense oligonucleotide, a phase separation technique is employed whereby a solution of the polymeric matrix material in a suitable organic solvent is prepared. To this solution is added the oligo suspended or dissolved in water or as fine particles alone. A non-solvent for the polymeric matrix material is slowly added to the stirred dispersion causing the polymeric material to slowly precipitate around the oligo forming microparticles. The microparticles are further hardened by the addition of a second non-solvent for the polymeric matrix material. The microparticles are then isolated by filtration and dried.

In other embodiments of the invention, the shaped nucleotide substance containing matrix material can assume forms other than microparticles such as rods, wafers, rectangularly shaped films or blocks. In each case the antisense oligonucleotide substance is distributed throughout the matrix material. The amount of oligo throughout the matrix is an amount sufficient to elicit the desired therapeutic response as the entrapped oligo substance is released by the implanted matrix material over an extended period of time. These shaped objects are particularly suitable for subcutaneous implantation into animals desired to be treated.

Yolles, in U.S. Pat. No. 3,887,699, discloses the preparation of drug-containing shaped polymeric objects of assorted shapes and sizes for subcutaneous implantation in a subject. This patent is incorporated herein by reference.

The shaped oligo containing matrix material can be administered in several ways. When the shaped matrix is in the form of microparticles, the most expedient mode of administration is by intramuscular injection, although microparticles can be subcutaneously implanted. For matrix material which is shaped into such objects as rods, wafers, films and the like, the most common and expedient mode of administration is by implantation.

## EXAMPLES

The following examples further describe the materials and methods used in carrying out the invention. The examples are not intended to limit the invention in any manner. The following terms have the following meanings: "sec" refers to second, "min" refers to minutes, "h" refers to hour, "d" refers to days, "mL" refers to milliLiter, "g" refers to gram.

## EXAMPLE 1

### Treatment of Tumors with CATO

The feasibility of controlled-release antisense technology with oligonucleotides (CATO) is tested for its ability to inhibit the expression of the c-myc proto-oncogene both *in vitro* in MDA-MB-231 cells and *in vivo* by treatment of nude mice bearing solid MDA-MB-231 human breast adenocarcinoma tumors. The particular CATO delivery system is designed for controlled release of the anti-c-myc oligonucleotide into the tumor cell over a 30 day time-frame.

Phosphorothioate oligodeoxyribonucleotides are the primary oligos used for antisense therapeutics. Oligos used for antisense inhibition of gene expression are designed and synthesized to complementarily hydrogen bond, i.e., a target gene sequence. One such sequence which has been shown to be sensitive to antisense therapy is the c-myc oncogene (Watson et al., Cancer Res., 51:3996-4000 (1991).

A. Preparation of Encapsulated Oligodeoxyribonucleotides

Synthethic anti-c-myc oligo is procured from a commercial source (Synthecell Corp., Rockville, MD). 100 mg of oligo is encapsulated in 1 g of 50:50 polylactide and polyglycolide (PLGA). The polymer is dissolved in ethyl acetate and encapsulated by the phase separation process (See U.S. Patent 4,919,929). Biodegradable polymer containing a mixture of 50% polylactic and 50% polyglycolic acids in a ratio of 65:35 (dl-PLGA) (Medisorb Technologies Int., Cincinnati, OH) is weighed into a 50 mL glass screw-cap tube and dissolved in 30 g of ethyl acetate and then poured into a 300 mL water jacketed reaction vessel cooled to 0°C. An additional 45 g of ethyl acetate is added to the reactor and the mixture is probe sonicated (Tekmar Model TM375) as 1 mL of DNA solution is slowly added using a 1 cc syringe and 18 gauge needle. After 30 sec of sonication, 75 g of 360 fluid 1000 cs (Dow Corning) silicon oil is added to the reactor over 2 min and this mixture is then immediately quenched by stirring at room temperature in 2.5 L of heptane (Chempure M138 KBJS). After 3.5 h the solid material is collected on a 0.2 um filter, washed with heptane and dried in a vacuum oven for 3 days. The microcapsules range in size from $\geq$1 µm to $\leq$250 µm. These microspheres are extremely sensitive to moisture and temperatures above 30°C and are therefore stored desiccated at 4°C.

B. In Vitro Test of Anti-c-myc CATO

Anti-c-myc CATO is first tested in vitro. It has been shown that treatment of MDA-MB-231 cells, in vitro, with anti-c-myc phosphorothioate oligos specifically inhibits the expression of the myc protein and inhibits cell growth (Watson et al., Cancer Res., 51:3996-4000 (1991)). Anti-c-myc CATO microspheres, free anti-c-myc

oligo, control CATO microspheres or control microspheres (no oligo) are incorporated into tissue culture medium containing 1 X $10^5$ MDA-MB-231 cells. The proliferation of the tumor cells is monitored by counting the cells at various time points after treatment with anti-c-myc oligo or with control oligo by the trypan blue dye viability exclusion method. Treated cells are harvested from the culture flask, spun down and diluted in 1 mL of fresh media. An aliquot of the cells is mixed with trypan blue stain, allowed to take up the stain for 5 min at room temperature and counted. C-myc mRNA levels are determined by reverse-transcriptase polymerase chain reaction (RT-PCR) analysis of total cellular RNA isolated from the treated cells. c-Myc protein expression is determined by immunocytochemical analysis of the treated and control cancer cells. Cells will be fixed and stained with fluorescent anti-c-myc antibody and observed to determine the amount of c-myc protein on the nuclear membrane. Applicants predict that this treatment of cancer cells will yield a reduction in the number of live cancer cells and the level of c-mycprotein and mRNA would be decreased.

C. In Vivo Test of Anti-c-myc CATO

In vivo studies are carried out in athymic nu/nu nude mice bearing MDA-MB-231 tumors. The mice are injected subcutaneously with MDA-MB-231 cells and tumors allowed to grow until palpable. The mice bearing tumors are injected with formulation containing 0.1, 0.5 or 5 mg of the anti-c-myc CATO microspheres intratumor (i.t.), free anti-c-myc oligo, control CATO microspheres i.t. or control microspheres (no oligo) i.t. The growth of the solid tumor is followed by palpation for 8 weeks or until the mice die. At the end of the study or when it is determined that the mice are near death, the mice are sacrificed and total cellular RNA is isolated from

the tumor cells for RT-PCR analysis of c-myc mRNA levels. Total cellular RNA from the tumors is purified and reverse transcribed to produce copy DNA (cDNA). The cDNA is amplified by PCR with oligonucleotide primers specific for c-myc sequence and quantitated following visualization by agarose/ethidium bromide gel electrophoresis. Tumor cells are also evaluated for c-myc protein expression by immunocytochemical analysis (as in (B) above). Mouse survival rate between treatment groups is also determined. Applicants predict that treatment with the therapeutic oligo will yield a reduction in size of palpable tumors in CATO-treated mice when compared to untreated mice. Additionally, Applicants predict that the amount of c-myc mRNA and protein in the tumor will be significantly reduced when compared to mice treated with the control oligo.

WHAT IS CLAIMED IS:

1. A microparticle composition suitable for the controlled release of an antisense oligodeoxyribonucleotide to a target cell, the microparticle composition comprising:

(a) an antisense oligodeoxyribonucleotide present in an amount of about 0.0001 wt % to 50.0 wt % of said antisense oligodeoxyribonucleotide based on the weight of an encapsulating matrix of element (b);

(b) a biodegradable polymeric matrix encapsulating the antisense oligodeoxyribonucleotide of elements (a); and

(c) the microparticle ranging in diameter from 1 to less than 500 microns.

2. The composition of Claim 1, wherein said antisense oligodeoxyribonucleotide is selected from the group consisting of phosphotriester, methylphosphonate, and alkyl phosphotriester, alpha-anomer, phophorothioate, diphosphorothioate, methylphosphorothioate, phosphodiester oligodeoxyribonucleotide.

3. The composition of Claim 2, wherein said antisense oligodeoxyribonucleotide is conjugated by way of chemical bonds with a material which promotes the uptake or transportation to the nucleus of the target cell of the antisense oligodeoxyribonucleotide, the material selected from the group consisting of proteins, intercalating agents, antibodies, cholesterol, cholesterol derivatives, lipids, nucleic acids and carbohydrates.

4. The composition of Claim 1, wherein said polymeric matrix material of said microparticle is selected from the group consisting of poly-d, l-lactic acid, poly-L-lactic acid, polyglycolic acid, copolymers

of mixed d, l-lactic acid and glycolic acid, copolymers of L-lactic acid and glycolic acid, copolyoxalates, polycaprolactone, poly (lactic acid-caprolactone), poly(glycolic acid-caprolactone), casein, albumin, waxes, polyanhydrides and polyorthoesters.

5. A method for the controlled delivery of the antisense oligonucleotide of Claim 1 into the target cells of an animal to alter gene expression, the method comprising:

(a) coating an antisense oligonucleotide onto a micro carrier of inert, dense material of a size less than the target cell size;

(b) encapsulating the coated microcarrier of step (a) within a biodegradable polymer matrix, the encapsulating step forming a microparticle composition;

(c) coating the microparticle composition of step (b) onto a planar carrier sheet, and

(d) discharging the microparticle composition with a force so that the carrier sheet is restrained from hitting the animal cells, but the microparticle composition is introduced into the target animal cell from which site it can interact with the nucleus of the target cell.

6. A method for the controlled delivery of an antisense oligodeoxyribonucleotide into the cells of an animal to alter expression of a target gene, the method comprising:

(a) encapsulating an antisense oligodeoxyribonucleotide within a biodegradable polymer matrix, the encapsulating step forming a microparticle composition;

(b) containing the microparticle composition of step (a) in a dosing device suitable for delivery of the microparticle to a target cell; and

(c) discharging the microparticle composition from the dosing device of step (a) into an animal from which site the antisense oligodeoxyribonucleotide contained within the biodegradable polymer matrix can interact with the target cell upon degradation of the polymer matrix.

7. A method for altering the expression of genes comprising:

introducing into a target animal tissue a controlled-release, biodegradable microparticle composition comprising:

(a) an antisense oligodeoxyribonucleotide specific to the gene expression to be inhibited and present in an amount of about 0.0001 wt % to 50 wt % based on the weight of an encapsulating polymeric matrix of element (b);

(b) a biodegradable polymeric matrix encapsulating the antisense oligodeoxyribonucleotide of element (a); and

(c) the microparticle ranging in diameter from 1 to 500 microns.

8. The method of Claim 7, wherein said antisense oligodeoxyribonucleotide is selected from the group consisting of phosphorothioates, phosphotriesters, methylphosphonate, alkyl phosphotriesters, alpha-anomer, diphosphorothioate, methylphosphorothioate, phosphodiester oligodeoxyribonucleotide.

9. The method of Claim 7, wherein said antisense oligdeoxyribonucleotides are conjugated by way of chemical bonds with molecules which promote the uptake into the cell and/or transportation to the nucleus, the molecules selected from the group consisting of fatty acids, phospholipids, sphingolipids, glycolipids, triglycerides, gangliosides, steroid hormones, cholesterol, peptidoglycans, lectins, single stranded or double stranded RNA, DNA, and intercalating agents.

10. The method of Claim 7, wherein said polymeric matrix containing the oligodeoxyribonucleotide is coated with molecules which promote the uptake of the nucleic acid into the cell and/or transportation to the nucleus, the molecule selected from a group consisting of proteins, intercalating agents, nucleic acids, lipids, or carbohydrates.

11. The method of Claim 7, wherein the polymeric matrix material of said microparticle is selected from the group consisting of poly-d, l-lactic acid, poly-l-lactic acid, polyglycolic acid, copolymers of mixed d,l-lactic acid and glycolic acid, copolymers l-lactic acid and glycolic acid, copolyoxalates, polycaprolactone, poly (lactic acid-caprolactone), poly (glycolic acid-caprolactone), casein, albumin, and waxes.

12. A method of controlling illness in animals that promotes uptake of an antisense oligonucleotide by a cell, the method comprising discharging a biodegradable microparticle composition of Claim 2 effective in inhibiting a specific disease into a target cell exhibiting that disease.

# INTERNATIONAL SEARCH REPORT

Int. ational application No.
PCT/US94/04287

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(5) :A61K 9/14, 9/16, 48/00; C07H 21/00, 21/04

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/44; 424/417, 420, 422, 425, 426, 482, 486, 489, 497, 498; 536/24.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | US, A, 4,945,050 (SANFORD ET AL.) 31 July 1990, see columns 7, 8, 10. | 5 |
| Y | EP, A2, 0,248,531 (TICE ET AL.) 12 September 1987, see entire document. | 1-12 |
| Y | Chemical Reviews, Volume 90, No. 4, issued June 1990, E. Uhlmann et al., "Antisense Oligonucleotides: A New Therapeutic Principle", pages 543-583, see entire document. | 1-12 |

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

20 JUNE 1994

Date of mailing of the international search report

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# INTERNATIONAL SEARCH REPORT

Inte. ional application No.
PCT/US94/04287

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | FEBS Letters, Volume 259, No. 2, issued January 1990, A. Kabanov et al., "A New Class of Antivirals: Antisense Oligonucleotides Combined with a Hydrophobic Substituent Effectively Inhibit Influenza Virus Reproduction and Synthesis of Virus-Specific Proteins in MDCK Cells", pages 327-330, see entire document. | 10 |
| A,P | Cancer Gene Therapy, Volume 1, No. 1, issued March 1994, B. Tseng et al., "Antisense Oligonucleotide Technology in the Development of Cancer Therapeutics", pages 65-71, see entire document. | 1-12 |
| Y,P | Science, Volume 261, issued 20 August 1993, C. Stein et al., "Antisense Oligonucleotides as Therapeutic Agents - Is the Bullet Really Magical?", pages 1004-1012, see entire document. | 1-12 |
| A | Anti-Cancer Drugs, Volume 3, issued 1992, R. Duncan, "Drug-Polymer Conjugates: Potential for Improved Chemotherapy", pages 175-210, see pages 204-205. | 1-12 |
| A | US, A, 4,389,330 (TICE ET AL.) 21 June 1983, see entire document. | 1-12 |
| A | US, A, 3,887,699 (YOLLES) 03 June 1975, see entire document. | 1-12 |
| A | US, A, 4,919,929 (BECK) 24 April 1990, see entire document. | 1-12 |
| A | US, A, 4,542,025 (TICE ET AL.) 17 September 1985, see entire document. | 1-12 |

# INTERNATIONAL SEARCH REPORT

Int ional application No.
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A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

514/44; 424/417, 420, 422, 425, 426, 482, 486, 489, 497, 498; 536/24.5

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, BIOSIS, MEDLINE, INPADOC
search terms: antisense, oligo?, encapsulat?, microencapsulat?, polymer?, matrix, lactic, lactide, glycolic, glycolide, copolymer?, polyoxalate, copolyoxalate, polyanhydride?, polyorthoester?, caprolactone?, polycaprolactone?, casein, albumin, wax